Chemical sensing based on membrane potential change induced by host-guest complexation at a membrane surface[1]

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Chemical sensing based on membrane potential change induced by host-guest complexation at a membrane surface[1]

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Three new principles for discrimination of organic guests by the membrane potential change are described, which are based on the host-guest recognition of charged groups, hydrogen bonding groups, or steric shapes of nonpolar moieties. (i) Potentiometric discrimination by the recognition of charged groups has been attained by liquid membrane type electrodes containing lipophilic macrocyclic polyamines as the sensory elements. These electrodes displayed potentiometric selectivities for organic anions according to the amount or proximity of negative charges. Thus, the strongest responses were observed for such guests as ATP^{4-} having a large number of negative charges as well as $maleate^{2-}$ and $phthalate^{2-}$ having negative charges at the closest distance within the molecule. (ii) Potentiometric discrimination by the recognition of hydrogen bonding groups has been attained by an electrode containing a lipophilic cytosine-pendant triamine, which functions as a ditopic receptor for guanine nucleotides by an electrostatic interaction with the phosphate group and a complementary base pairing with the guanine base. This electrode displayed nearly all-or-nothing selectivities between guanine and adenine nucleotides. Selective responses to guanine nucleotides were also obtained by double receptor type electrodes containing a macrocyclic tetraamine and a neutral cytosine derivative despite the fact that both of these hosts, when existed separately, showed only weak responses to these guests. (iii) Potentiometric discrimination by the recognition of steric shapes of nonpolar moieties has been attained by an electrode containing a lipophilic hexaester of calix[6]arene having an inclusion cavity for organic guests. At pH 7.0, the strongest responses were obtained for primary amine guests such as 2-phenylethylamine and 1-octylamine having no bulky substituent at the α-carbon that could sterically hinder the formation of tripodal hydrogen bonds between the NH_3^+ group of the guest and the ester carbonyl groups of the host. A comparison of potentiometric selectivities with those of the electrode containing dibenzo-18-crown-6, as well as ^1H-NMR results on the geometry of the host-guest complex, strongly supported a potentiometric shape discrimination effect based on the inclusion of guests within the calix[6]arene cavity having a well-defined structure.

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INTRODUCTION

Host-guest molecular recognition, occurring either in solutions or at membrane surfaces, is a fundamental chemical process that controls many important biological reactions such as antigen-antibody reactions, enzymatic reactions, carrier- or channel-mediated transport, and receptor reactions involving signal transduction (Figure 1).[2] From the viewpoint of analytical chemistry, the function of signal transduction at biomembranes gives important implications for the sensing of target chemical substances. There are two representative modes of signal transduction that could be induced by host-guest complexation at membrane surfaces, *i.e.*, the membrane potential change and the membrane permeability change.[3]

Figure 1 Biological functions based on host-guest complexation in solutions or at membrane surfaces. (a) Separation of guests as in antigen-antibody reactions. (b) Chemical transformation of guests as in enzymatic reactions. (c) Translocation of guests as in carrier- or channel-mediated transports. (d) Signal transduction by complexation with guests as in receptor reactions.

Of these two modes, the guest-induced membrane potential change is the principle of signal transduction that has been extensively exploited for ion-selective electrodes (ISEs), which consist an important method for *in situ* analysis of target chemical substances.[4] The electrode system that is most wid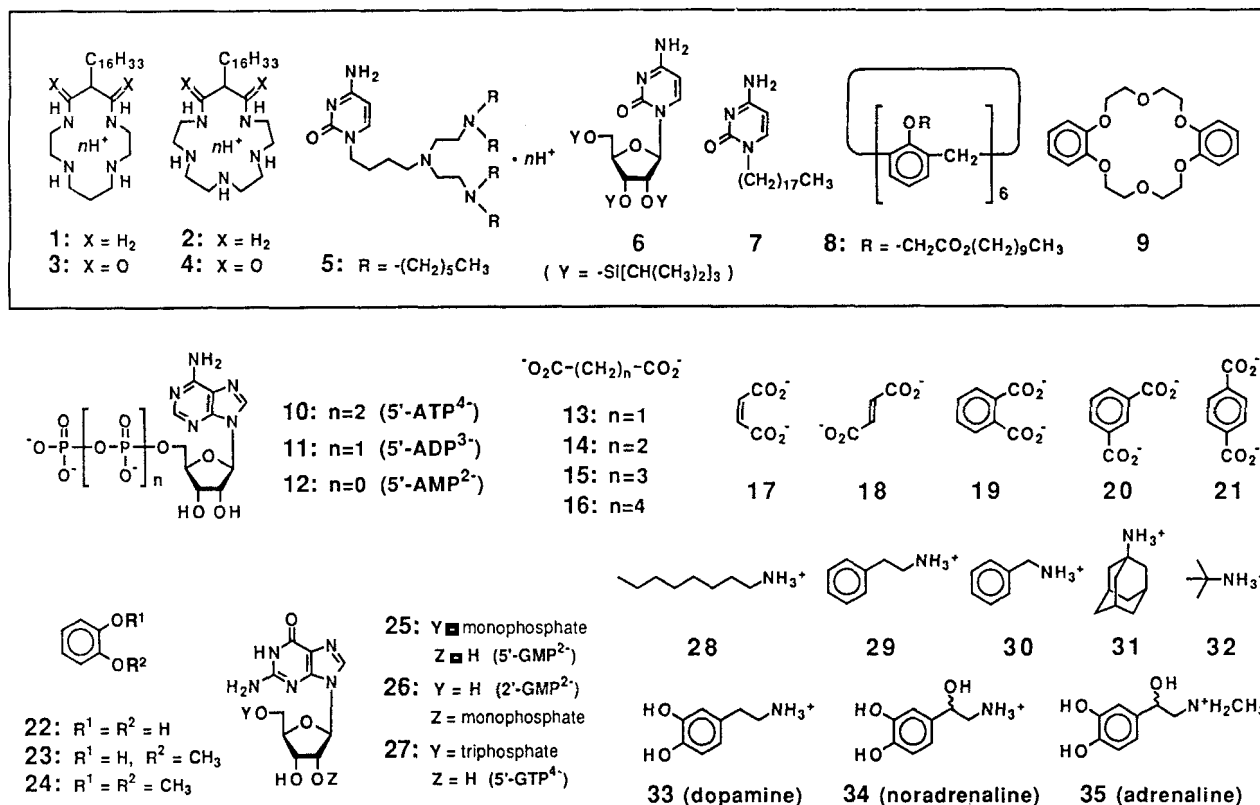ely used is a liquid membrane type, in which a lipophilic organic liquid membrane containing lipophilic host molecules is generally supported by a poly(vinyl chloride) (PVC) polymer matrix. The fundamental chemical process occurring in liquid membrane ISEs is a guest-induced selective change in the charge separation across the interface between the liquid membrane and the aqueous sample solution (*vide infra*). This sort of chemical process is a phenomenon that is unique to membrane interfaces.

Recent advances in the host-guest chemistry for the design and synthesis of artificial receptor molecules have stimulated a number of research groups to develop a novel type of liquid membrane ISEs where synthetic host molecules with specific receptor functions are used as sensory elements. Many electrodes with excellent selectivities for inorganic cations have been developed by using totally synthetic hosts such as crown compounds and acyclic ligands.[4] On the other hand, there have been only limited examples of electrodes that strongly respond to and discriminate between organic guests. The main principles employed so far for these electrodes are the potentiometric discrimination based on lipophilicity[5] or chirality[6] of organic guests. In this paper, we present

three new principles for discrimination of organic guests by the membrane potential changes induced by host-guest complexation at the membrane surface. The first two principles are the potentiometric discrimination based on the interaction with specific functional groups of organic guests, such as charged groups or hydrogen bonding groups. The third principle is the potentiometric discrimination based on the recognition of the steric shapes of nonpolar moieties of organic guests by inclusion into a cavity of a host having a well-defined structure. The hosts (1~9) and organic guests (10~35) used in the present study are shown in Figure 2.

RESULTS AND DISCUSSION

Potentiometric Discrimination of Organic Guests Based on the Recognition of Charged Groups. The recognition of charged groups could be an important principle for potentiometric discrimination of organic guests because it generally involves strong electrostatic interactions between distinct sites in the molecule. The capability of such a mode of potentiometric discrimination has been demonstrated by a series of studies on anion-selective electrodes using lipophilic derivatives of macrocyclic polyamines as the sensory elements.[7a-c] Macrocyclic polyamines acquire a strong anion receptor function by multiple protonation in acidic to neutral aqueous solutions.[8-11] Their complexation selectivities-

1: X = H$_2$ **2:** X = H$_2$
3: X = O   **4:** X = O   **5:** R = -(CH$_2$)$_5$CH$_3$ **6**   **7**   **8:** R = -CH$_2$CO$_2$(CH$_2$)$_9$CH$_3$ **9**
(Y = -Si[CH(CH$_3$)$_2$]$_3$)

10: n=2 (5'-ATP^{4-})
11: n=1 (5'-ADP^{3-})
12: n=0 (5'-AMP^{2-})

^-O_2C-(CH$_2$)$_n$-CO$_2^-$
13: n=1
14: n=2
15: n=3
16: n=4

17   18   19   20   21

28   29   30   31   32

22: R^1 = R^2 = H
23: R^1 = H, R^2 = CH$_3$
24: R^1 = R^2 = CH$_3$

25: Y■ monophosphate Z□ H (5'-GMP^{2-})
26: Y = H (2'-GMP^{2-}) Z = monophosphate
27: Y = triphosphate Z = H (5'-GTP^{4-})

**33 (dopamine)   34 (noradrenaline)   35 (adrenaline)**

Figure 2 Structures of the hosts (1~9) and organic guests (10~35) used in the present study. The hosts and guests are shown in their predominant charged forms under the experimental conditions.

generally reflect the electrostatic interaction between the host and guest.[8-11] Accordingly, lipophilic derivatives of macrocyclic polyamines, which are soluble in lipophilic liquid membranes and capable of multiple protonation at the membrane surface by the uptake of protons from the aqueous sample solution, can be used as sensory elements for anion-selective electrodes.

(a) Stepwise Protonation Behaviors at the Membrane Surface. Lipophilic derivatives of macrocyclic tetra- and pentaamines (1, 2) were synthesized and used as the sensory elements of PVC matrix liquid membrane electrodes (electrodes 1 and 2, respectively).[7a-c] The stepwise protonation property of macrocyclic polyamines results in a pH dependent magnitude of positive charge which affects the membrane potential of the electrode. Figure 3 shows pH vs potential curves for electrode 2 in the presence and absence of maleic acid (17), which could exist mainly as its mono- or dianionic form depending on the pH (pK$_1$ and pK$_2$ of guest 17 are 1.94 and 6.22, respectively). In the absence of the guest, the membrane potential increased with decreasing pH. Upon an addition of the guest, the pH-potential curve deviated from that in the absence of the guest below ca. pH 8.5, showing a negative potential shift (anionic potentiometric response).[7b]

Figure 4 shows a possible mechanism that leads to the anionic potentiometric response. By the multiple proton
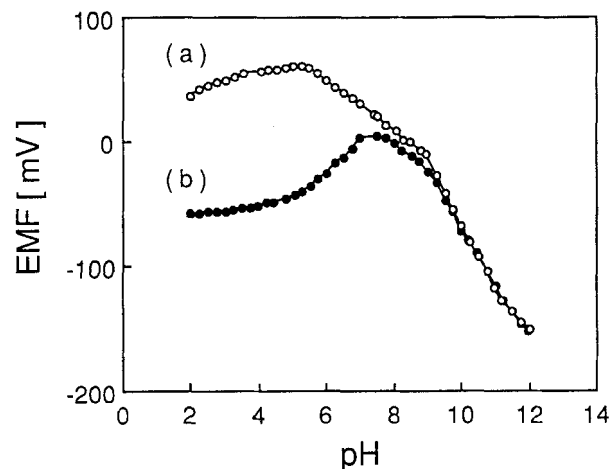
Figure 3 Potential vs pH curves in the presence and absence of maleic acid (17), obtained by electrode 2 containing macrocyclic pentaamine 2 as a sensory element. (a) Curve in the absence of guest 17. The initial condition was 0.01 M H$_2$SO$_4$. The pH was adjusted by an addition of an aq solution of NaOH containing 0.01 M Na$_2$SO$_4$. (b) Curve in the presence of guest 17. The initial condition was 0.01 M H$_2$SO$_4$ + 0.01 M of guest 17. The pH was adjusted by an addition of an aq solution of NaOH containing 0.01 M Na$_2$SO$_4$ and 0.01 M of guest 17.[7b]
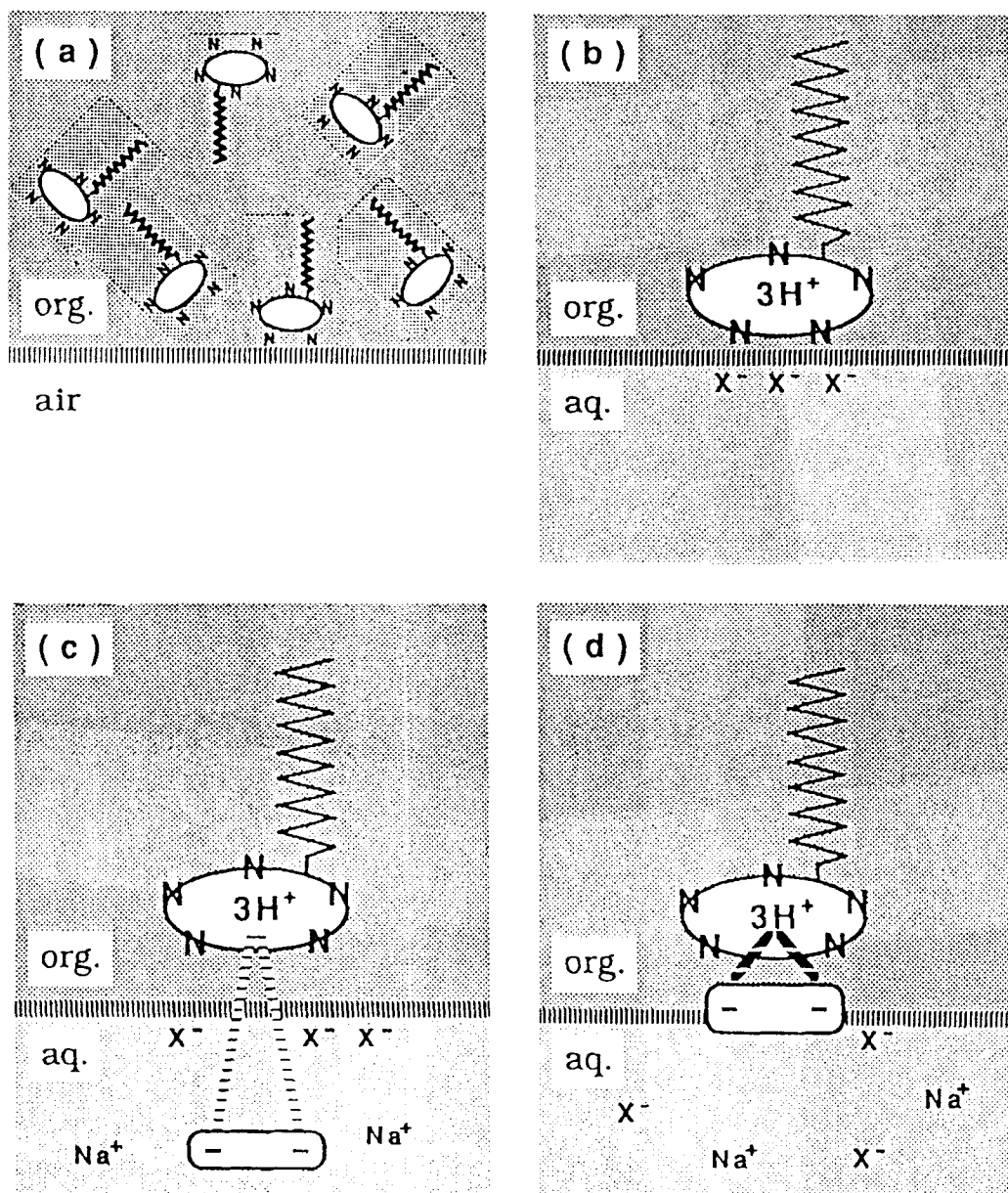
Figure 4 A possible mechanism of potentiometric response to anionic guests. (a)→(b): Proton uptake by macrocyclic polyamines at the membrane surface, inducing a charge separation that leads to a positive membrane potential. (c)→(d): Host-guest complex formation and the consequent decrease in the charge separation, resulting in an anionic potentiometric response.[3b,c,7b]

uptake, a charge separation between the protonated lipophilic polyamine and its hydrophilic counter anion occurs at the membrane interface to generate a positive membrane potential (Figure 4a → 4b). At the same time, the multiply protonated host becomes capable of complexing with anionic guests. The charge separation as well as the anion receptor function would increase with decreasing pH. Then, the host-guest complexation with an organic anion, which entails an exchange of the hydrophilic counter anion to a more lipophilic one, would cause a decrease in the charge separation, resulting in an anionic potentiometric response (Figure 4c → 4d).[3b,c,7b]

The disappearance of potentiometric responses in the higher pH region reflects the loss of host functionality of macrocyclic polyamines due to an insufficient protonation.[7a-c]

Potentiometric response behaviors of electrodes 1 and 2 have been examined for a series of adenine nucleotides (10~12) and three series of dicarboxylate guests (13~16; 17, 18; 19~21). In all cases, the membrane potential decreased with an increasing concentration of the guest. Figure 5 shows, as a typical example, potential vs concentration curves (potentiometric response curves) for guests 17 and 18, observed by electrode 2 as well as by

Table 1 Potentiometric Selectivity Coefficients ($K^{pot}_{i,j}$) of the Macrocyclic Pentaamine Electrode (Electrode 2) for Nucleotide and Dicarboxylate Guests[a]

Guest	$KP^{pot}_{i,j}$	Guest	$KP^{pot}_{i,j}$
Adenine nucleotides[b]		**Geometrical isomers**[d]	
10 (ATP⁴⁻)	1	17 (*cis*)	1
11 (ADP³⁻)	0.035	18 (*trans*)	0.08
12 (AMP²⁻)	0.030		
Linear homologues[c]		**Positional isomers**[e]	
13 (n = 1)	1	19 (*ortho*)	1
14 (n = 2)	0.78	20 (*meta*)	0.19
15 (n = 3)	0.74	21 (*para*)	0.04
16 (n = 4)	0.68		

[a] Membrane composition: 2:DOP:PVC = 3:75:22. DOP = dioctyl phthalate. See refs 7a,b for the details.
[b] Determined in 10 mM HEPES-NaOH buffer (pH 6.7) at room temperature (*ca.* 20 °C) by the matched potential method according to ref 26.[7a] HEPES = *N*-(2-hydroxylethyl)piperazine-*N*'-2-ethanesulfonic acid.
[c] Determined in 5 mM HEPES-NaOH buffer (pH 7.7) at room temperature (*ca.* 20 °C) by the separate solution method according to ref 27.[7b]
[d] Determined in 5 mM HEPES-NaOH buffer (pH 8.2) at room temperature (*ca.* 20 °C) by the separate solution method according to ref 27.[7b]
[e] Determined in 5 mM HEPES-NaOH buffer (pH 7.4) at room temperature (*ca.* 20 °C) by the separate solution method according to ref 27.[7b]
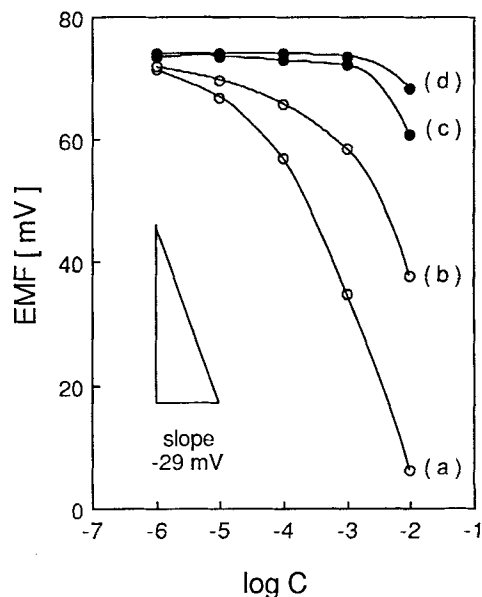
an electrode containing Capriquat® (methyltrioctylammonium chloride, >86%) as a conventional anion exchanger.[7b]

(b) Potentiometric Discrimination between AMP, ADP, and ATP Based on the Amount of Negative Charges. Potentiometric responses of electrodes 1 and 2 to a series of adenine nucleotides, ATP (**10**), ADP (**11**) and AMP (**12**), have been measured at pH 6.7, under which the guests exist mainly as ATP⁴⁻, ADP³⁻, and AMP²⁻, respectively.[7a,c] Especially by electrode 2, excellent sensitivity and selectivity for ATP polyanion was attained. A linear response was obtained for log[ATP] in a concentration range between $10^{-3} \sim 10^{-7}$ M with a slope of -14.5 mV/decade (*ca.* 20 °C), which is in good agreement with the theoretical value for a tetravalent anion according to the Nernst equation. The response to ATP⁴⁻ was by far the strongest when compared with the other guests. The potentiometric selectivity coefficients ($K^{pot}_{i,j}$) for electrode 2 are listed in Table 1[7a]. The order of the response was ATP⁴⁻ >> ADP³⁻ ≥ AMP²⁻, corresponding to the amount of electrostatic interaction between the protonated macrocyclic polyamine and the anionic guest.

(c) Potentiometric Discrimination between Dicarboxylate Guests Based on the Proximity of Negative Charges. Discrimination of anionic guests having the same amount of charge but with different positional relations of the anionic groups within the molecule is displayed by protonated macrocyclic polyamines in water.[10a,11a,c] In this context, potentiometric discrimination of three series of dicarboxylate guests with different distances between the two negative charges has been

systematically examined for electrodes 1 and 2.[7b,c] The three series of dianionic guests examined are linear homologues (**13~16**), and geometrical (**17**, **18**) and positional (**19~21**) isomers of dicarboxylates. The pH condition was carefully set for each series of guests to specifically observe potentiometric responses to their *dianionic* forms.

Potentiometric selectivity coefficients ($K^{pot}_{i,j}$) for electrode 2 are listed in Table I.[7b] For the linear homologues of terminal dicarboxylates (**13~16**), a small potentiometric discrimination was observed with a response order of **13** (n = 1) > **14** (n = 2) ≥ **15** (n = 3) ≥ **16** (n = 4), decreasing in the order of increasing distance between the two carboxylate groups. For the geometrical isomers of dicarboxylates, *i.e.*, maleate (**17**) and fumarate (**18**), a much stronger potentiometric response was observed for **17** (*cis*) than for **18** (*trans*) (Figure 5). Thus, the greater response was obtained for the isomer with a shorter distance between the two negative charges. In addition, the potentiometric discrimination between these rigid guests was much greater than that between the flexible linear homologues (**13~16**). For the positional isomers of dicarboxylates, *i.e.*, phthalate (**19**), isophthalate (**20**) and terephthalate (**21**), potentiometric discrimination was observed with a response order of **19** (*ortho*) > **20** (*meta*) > **21** (*para*). Again, the magnitude of the potentiometric response was in the order of decreasing distance between the two negative charges within the guest molecule.[7b] A comparison between electrodes 1 and 2 revealed that the

Figure 5 Potential vs concentration curves obtained for maleate (**17**) and fumarate (**18**) at pH 8.2. Curves obtained by electrode 2 containing macrocyclic pentaamine **2** (○) as well as by an electrode containing Capriquat® (●) are shown. Curves (a) and (c), and (b) and (d) correspond to the *cis* (**17**) and *trans* (**18**) isomers, respectively. Measured in 5 mM HEPES-NaOH buffer (pH 8.2) at room temperature (*ca.* 20 °C).[7b]
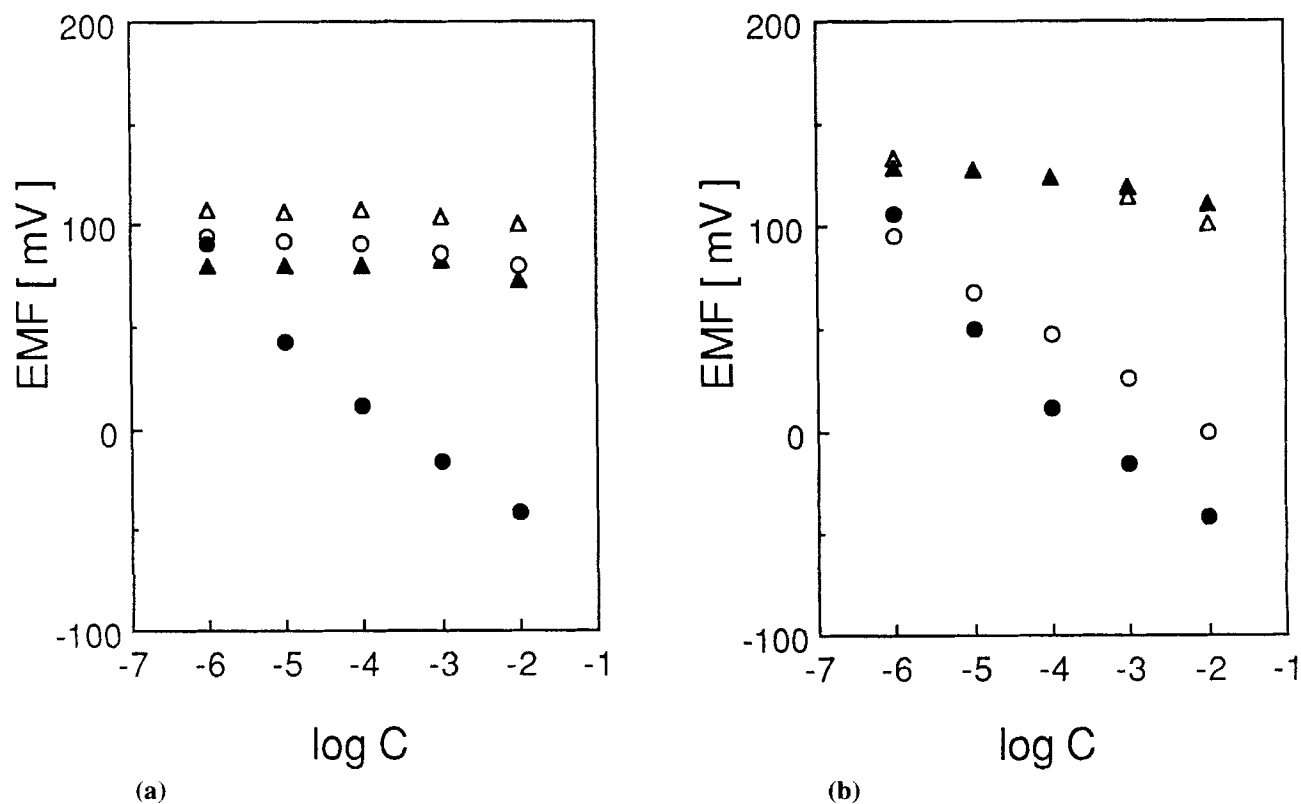
latter electrode containing macrocyclic pentaamine (**2**), which has a stronger cationic property due to its higher degree of protonation at the same pH, showed stronger potentiometric responses as well as higher selectivities for both nucleotide and dicarboxylate guests.[7c] The potentiometric selectivities for the nucleotide and dicarboxylate guests clearly reflect the general complexation selectivities displayed by protonated macrocyclic polyamines in water.[8–11]

(d) **Comparison of Macrocyclic Polyamines with Other Sensory Elements.** Although a liquid membrane electrode containing Capriquat®, a conventional anion exchanger, also showed some potentiometric discrimination between guests **17** and **18**, electrode 2 containing macrocyclic polyamine **2** as the sensory element was superior in every aspects, *i.e.*, selectivity, sensitivity, dynamic range, and response time (Figure 5).[7b] Compared to other nonconventional sensory elements for which potentiometric responses to organic anions have been reported (*e.g.*, derivatives of vitamin B_{12} and metalloporphyrin,[12] and diphosphonium and bis(quaternary ammonium) salts[13]), the macrocyclic polyamine electrodes displayed much better potentiometric selectivities to organic anions.[7a–c] Consequently, the macrocyclic polyamine

electrodes have disclosed an important principle for potentiometric discrimination of organic guests, *i.e.*, the recognition of the amount or proximity of negative charges by strong electrostatic interactions.

(e) **Potentiometric Responses to Other Types of Guests.** The macrocyclic polyamine electrode displayed potentiometric responses not only to anionic guests but also to neutral guests having hydrogen bonding groups. A strong anionic response has been unexpectedly observed by electrode 2 for catechol (**22**, $pK_1 = 9.5$) at pH 6.1 despite the fact that, in the bulk aqueous phase of this pH, the guest is almost completely in its uncharged neutral form. In contrast, the response to the monomethoxy derivative **23** with only one OH group was much smaller, and that to the dimethoxy derivative **24** without any OH group was negligible.[14]

On the basis of a systematic investigation with related neutral guests, we proposed a proton ejection mechanism, which involves an ejection of protons from the organic to aqueous phase as a result of strong hydrogen bonding interactions between the protonated macrocyclic polyamine and the hydroxyl groups of these guests at the membrane surface. This could be a simple plausible mechanism for the "anionic potentiometric responses" to

Figure 6 Potential vs concentration curves for anionic metal cyano complexes at pH 4.0. (a) Electrode 3 containing macrocyclic dioxotetraamine **3** as a sensory element. (b) Electrode 4 containing macrocyclic dioxopentaamine **4** as a sensory element. Measured in 0.01 M AcONa-AcOH buffer (pH 4.0) at room temperature (*ca.* 20°C). The guest anions tested were $[Ni(CN)_4]^{2-}$ (●), $[Fe(CN)_5(NO)]^{2-}$ (○), $[Fe(CN)_6]^{3-}$ (▲), and $[Fe(CN)_6]^{4-}$ (△).

the neutral guests.[14] These results seem to be consistent with the previous findings[15] that a protonated macrocyclic polyamine forms 1:1 complexes in neutral water with catechol and related guests. Although the induction of a change in the net charge separation and, consequently, membrane potential by uncharged neutral species is a puzzling problem, these findings gives an implication for a possibility of potentiometric discrimination of organic guests on the basis of hydrogen bonding.
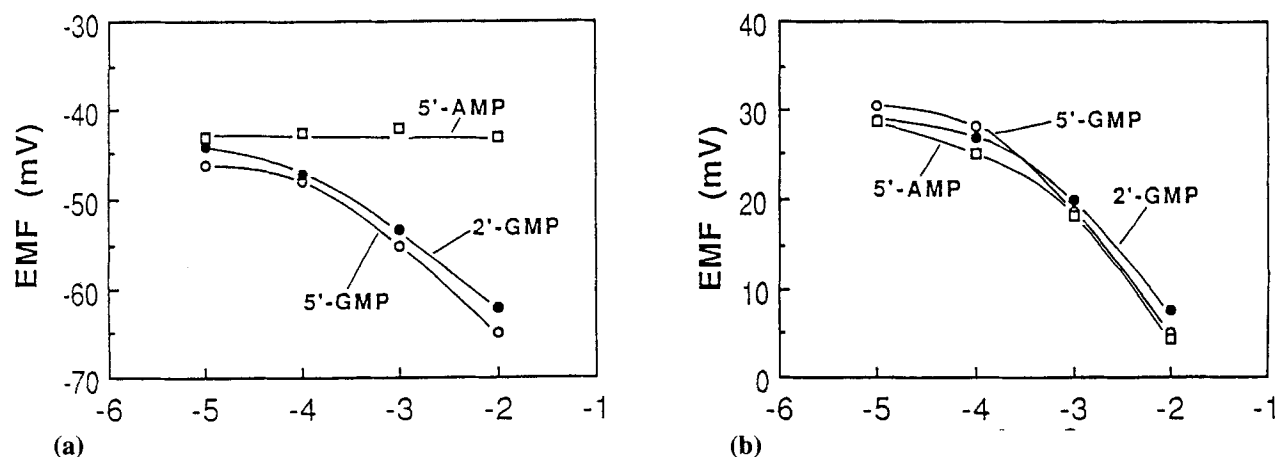
Another interesting potentiometric response behaviors have been observed for anionic metal cyano complexes by an electrode containing macrocyclic dioxotetraamine 3 as a sensory element (electrode 3).[16] Figure 6a shows potentiometric response curves for anionic metal cyano complexes having a square planar or octahedral structure. Electrode 3 showed an anionic response to nickel tetracyano complex, which has a square planar structure. On the other hand, the other anionic metal cyano complexes having an octahedral structure did not give any appreciable response. The same tendency, though not as distinct as in electrode 3, was observed for an electrode containing macrocyclic dioxopentaamine 4 (electrode 4) as shown in Figure 6b. Although a detailed analysis is in progress, these findings give an implication for a possibility of potentiometric discrimination on the basis of shape recognition.

Potentiometric Discrimination of Organic Guests Based on the Recognition of Hydrogen Bonding Groups. The recognition of an array of hydrogen bonding sites could be another important principle for potentiometric discrimination of organic guests because it generally involves directed interactions that are moderately strong. This mode of potentiometric discrimination has been demonstrated by the studies on PVC matrix liquid membrane electrodes containing a host with a ditopic

receptor function (5)[17] or a mixture of hosts for an electrostatic binding and a complementary base pairing (1 or 3 + 6).[18]
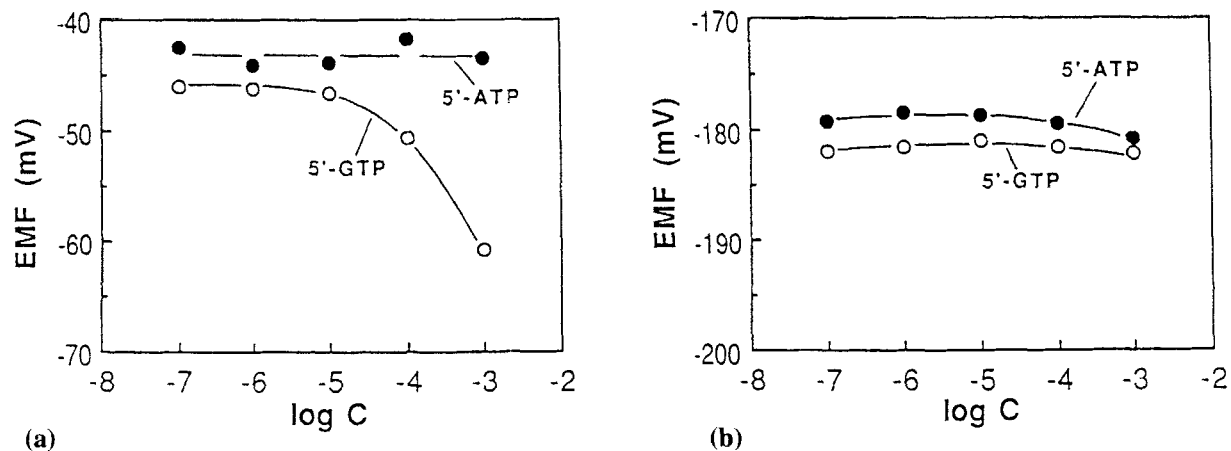
(a) Ditopic Receptor Type Electrode. The cytosine-pendant triamine host (5) is characteristic in that it has ditopic recognition sites for guanine nucleotides (25~27). The triamine moiety, in a protonated form, functions as an electrostatic binding site for the phosphate group, and the appended cytosine moiety as a complementary base pairing site for the guanine base.[19] The potentiometric response behaviors of the liquid membrane electrode containing host 5 as the sensory element (electrode 5) have been compared with those of the electrodes containing macrocyclic pentaamine 2 (electrode 2) or neutral cytosine derivative 6 or 7 (electrodes 6 and 7, respectively).[17]

At pH 6.6, the electrode based on host 5 (electrode 5) showed anionic potentiometric responses to 5'-GMP (25) and 2'-GMP (26) but not to 5'-AMP (12) (Figure 7a). A complete potentiometric discrimination was observed. On the other hand, the electrode based on macrocyclic pentaamine 2 gave strong responses to both GMP and AMP without any appreciable discrimination (Figure 7b). A complete potentiometric discrimination was also observed between 5'-GTP (27) and 5'-ATP (10). As shown in Figure 8a, electrode 5 displayed a moderately strong response to 5'-GTP (27) at pH 6.6 from ca. 10^{-5} M concentration. On the other hand, no response was observed for 5'-ATP (10) (Figure 8a) despite the expectation that, under this pH, 5'-ATP exists mainly as its tetraanionic form and hence must interact with the host more strongly than 5'-AMP (12) if the electrostatic interaction was considered. In the case of electrodes 6 and 7 containing neutral cytosine derivatives (6, 7) that lack an amine moiety as an electrostatic binding site, no appre-

-30

-70

-6   -5   -4   -3   -2   -1

(a)

5'-AMP

2'-GMP

5'-GMP

EMF (mV)

40

0

-6   -5   -4   -3   -2   -1

(b)

5'-GMP

5'-AMP

2'-GMP

EMF (mV)

Figure 7 Potential vs concentration curves for 5'-GMP (25), 2'-GMP (26), and 5'-AMP (12) at pH 6.6. (a) Electrode 5 containing the ditopic receptor 5 as a sensory element. (b) Electrode 2 containing macrocyclic pentaamine 2 as a sensory element. Measured in 0.1 M HEPES-NaOH buffer (pH 6.6) at room temperature (ca. 20°C).[17]

Figure 8 Potential vs concentration curves for 5'-GTP (27) and 5'-ATP (10) at pH 6.6. (a) Electrode 5 containing the ditopic receptor 5 as a sensory element. (b) Electrode 7 containing neutral cytosine derivative 7. Measured in 0.1 M HEPES-NaOH buffer (pH 6.6) at room temperature (ca. 20°C)[17].

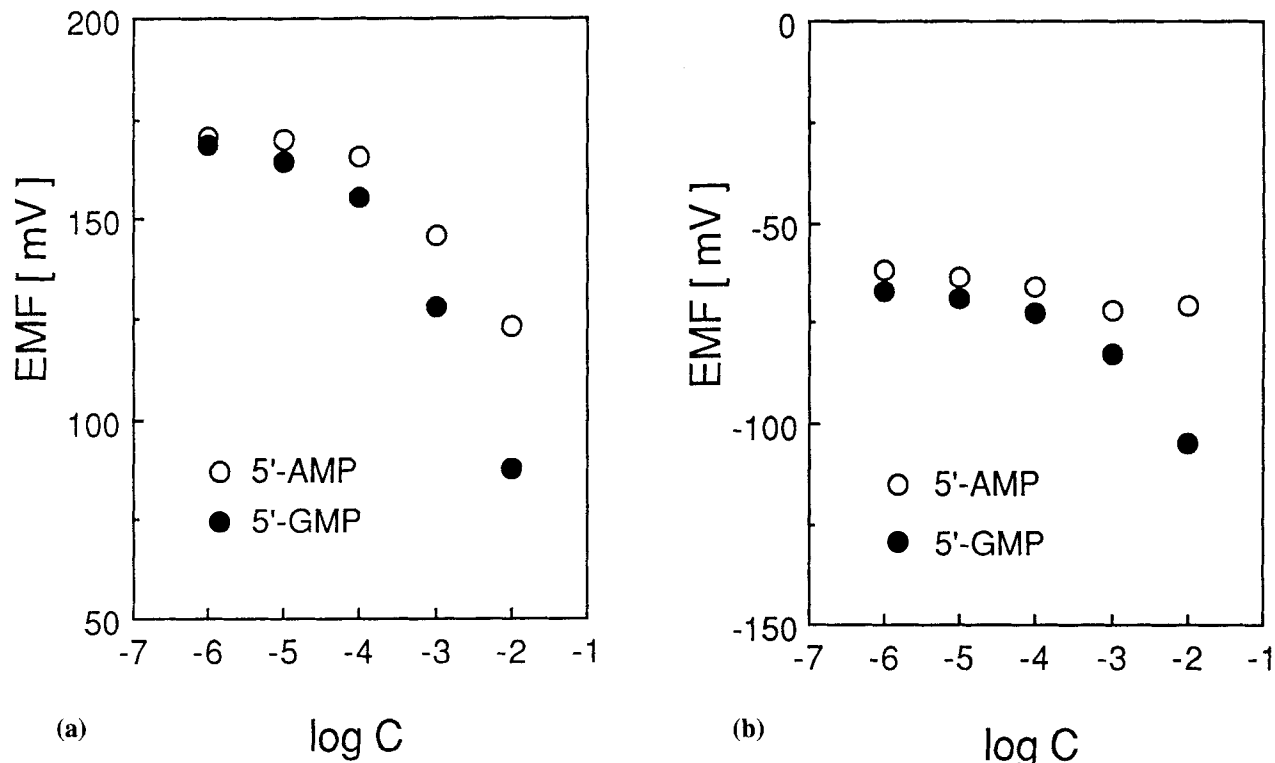ciable potentiometric response was observed either for 5'-GTP (27) or 5'-ATP (10) (Figure 8b).[17]

Thus, a complete potentiometric discrimination between guanine and adenine nucleotides has been attained by electrode 5 on the basis of moderate responses to the guanine nucleotides as compared to no response to the adenine nucleotides. The key point seems to be a moderately weak anion receptor function of the triamine host (5) as compared to the pentaamine host (2) which showed strong responses to both guanine and adenine nucleotides. On the other hand, no appreciable response was obtained by neutral cytosine derivatives 6 and 7 having a base pairing site but no electrostatic binding site. These results, taken together, indicate a characteristic feature of the cytosine-pendant triamine host (5); this host is only able to effect a potentiometric response to nucleotides when a complementary base pairing is possible in addition to a moderately weak electrostatic interaction.[17] This aspect can be regarded as an efficient cooperative effect leading to nearly all-or-nothing discrimination.

(b) Double Receptor Type Electrode. In order to gain a further insight into this kind of cooperative effect, we have examined double receptor type liquid membrane electrodes containing two kinds of host, *i.e.*, a saturated or dioxo type macrocyclic tetraamine (1 or 3) and a neutral cytosine derivative (6) (electrodes 1+6 and 3+6, respectively).[18] The polyamine host (1 or 3) is for a moderately weak electrostatic interaction in its protonated form, and the cytosine derivative (6) for a selective base pairing with guanine nucleotides. Both of these hosts, when existed separately, show only very weak potentiometric responses to 5'-GMP (25) or 5'-AMP (12). In fact, the tetraamine host did not show any appreciable response to 5'-AMP at pH 6.7, although a strong response to 5'-AMP was observed under the same condi-

tion by the corresponding pentaamine host (2) due to its higher degree of protonation.[7c] Also, as described above, the lipophilic neutral cytosine derivative (6) showed only very weak potentiometric responses to 5'-GMP or 5'-AMP at pH 6.6 without any discrimination[18] despite the expectation from its selective carrier function displayed for guanosine in the transport through a chloroform liquid membrane.[20]

However, when these two kinds of host coexist in the liquid membrane as in the cases of electrodes 1+6 and 3+6, potentiometric responses that are selective to 5'-GMP (25) have been observed. Whereas electrode 1+6 showed responses to both 5'-GMP (25) and 5'-AMP (12) (Figure 9a), electrode 3+6 containing host 3 which is a weaker anion receptor as compared to host 1 showed a response only to 5'-GMP (25) with no appreciable response to 5'-AMP (12) (Figure 9b).[18] These results would be explained by the feasibility of a ditopic recognition by two separate hosts, which leads to the formation of lipophilic ternary complexes composed of the two hosts and 5'-GMP (25). Such a potentiometric discrimination of organic guests based on a cooperative effect is still a rare example in the area of ion-selective electrodes, but is expected to be a new and versatile principle for potentiometric discrimination of organic guests.

Potentiometric Discrimination of Organic Guests Based on the Recognition of Steric Shapes of Nonpolar Moieties. The recognition of the steric shapes of nonpolar moieties could be a general principle for potentiometric discrimination of organic guests because organic guests generally contain nonpolar moieties having specific steric structures. Such a mode of potentiometric response has been demonstrated by an investigation on the response behaviors of a PVC matrix liquid membrane electrode containing as the sensory element a lipophilic derivative of calix[6]arene (8) that has an in-

(a)

200

150

EMF [mV]

100

O 5'-AMP
● 5'-GMP

50

-7 -6 -5 -4 -3 -2 -1

log C

(b)

0

-50

EMF [mV]

-100

O 5'-AMP
● 5'-GMP

-150

-7 -6 -5 -4 -3 -2 -1

log C

Figure 9 Potential vs concentration curves for 5'-GMP (**25**) and 5'-AMP (**12**) obtained at pH 6.7 by double receptor type electrodes. (a) Electrode 1+6 containing macrocyclic tetraamine **1** and neutral cytosine derivative **6** as sensory elements. (b) Electrode 3+6 containing macrocyclic dioxotetraamine **3** and neutral cytosine derivative **6** as sensory elements. Measured in 0.01 M HEPES-NaOH buffer (pH 6.7) at room temperature (ca. 20°C)[18].

clusion cavity for organic guests.[21] Recently, increasing attention has been focused on calixarenes as a class of hosts having well-defined cavities.[22] This class of hosts,

Table II Potentiometric Selectivity Coefficients ($K^{pot}_{i,j}$) of the Calixarene Electrode (Electrodes 8) and the Crown Ether Electrode (Electrode 9) for Simple Primary Amines and Catecholamine Guests at pH 7.0[a]

Guest[b]	Electrode 8		Electrode 9	
	Method A[c]	Method B[d]	Method A[c]	Method B[d]
Simple primary amines[e]				
28	2.60	1.41	20.0	20.0
29	1	1	1	1
30	2.90×10^{-1}	7.76×10^{-2}	4.77	4.70×10^{-1}
31	$< 10^{-2}$	1.55×10^{-3}	20.0	2.86
32	$< 10^{-2}$	7.41×10^{-5}	3.48×10^{-1}	2.69×10^{-2}
Catecholamines[f]				
33	1	1	1	1
34	1.99×10^{-1}	3.80×10^{-2}	9.35×10^{-1}	2.20×10^{-1}
35	2.69×10^{-1}	2.82×10^{-2}	8.82×10^{-1}	8.13×10^{-2}

[a] Membrane compositions of electrodes 8 and 9: 8:DOS:PVC = 5:68:27; 9:DOS:PVC = 2:66:32. DOS = dioctyl sebacate (bis(2-ethylhexyl) sebacate). See ref 21 for the details.
[b] All of the guests are almost completely in their monocationic forms by protonation under the experimental pH.
[c] Matched potential method.
[d] Separate solution method.
[e] The $K^{pot}_{i,j}$ values were determined at room temperature (ca. 20°C) in 0.1 M Tris-HCl buffer (pH 7.0). Tris = tris(hydroxymethyl)aminomethane.
[f] The $K^{pot}_{i,j}$ values were determined at room temperature (ca. 20°C) in 0.1 M Tris-HCl buffer containing 0.01 M L-ascorbic acid (pH 7.0).

especially calix[4]arenes, have been recently exploited as sensory elements of ion-selective electrodes, but all of the studies carried out so far are limited to electrodes for inorganic metal cations.[23]

As a new sensory element for organic guests, we have synthesized a lipophilic derivative of calix[6]arene (**8**) which is completely derivatized with acetic acid esters on the lower rim.[21] As shown in Figure 10a, when host **8** adopted the cone conformation, the cavity of calix[6]arene would be sufficiently large for inclusion of organic guests. In addition, the ester carbonyl groups that are directed inward would afford a convergent binding site like 18-crown-6, which has an ideal arrangement of ethereal oxygen atoms for a tripodal hydrogen bonding with protonated primary amines (Figure 10b).

Potentiometric responses of the electrode containing calix[6]arene hexaester **8** as the sensory element (electrode 8) have been examined for a series of simple primary amines (**28~32**) at pH 7.0, at which these guests are almost completely in their monocationic forms by the protonation. Figures 11a and 11b show the potentiometric response curves for these guests, observed by electrode 8 as well as by an electrode containing dibenzo-18-crown-6 (**9**) as a sensory element (electrode 9). The potentiometric selectivity coefficients ($K^{pot}_{i,j}$) are listed in Table II. As shown in Figure 11b and Table II, electrode 9 showed a selectivity which was evidently con-

## (a)   (b)
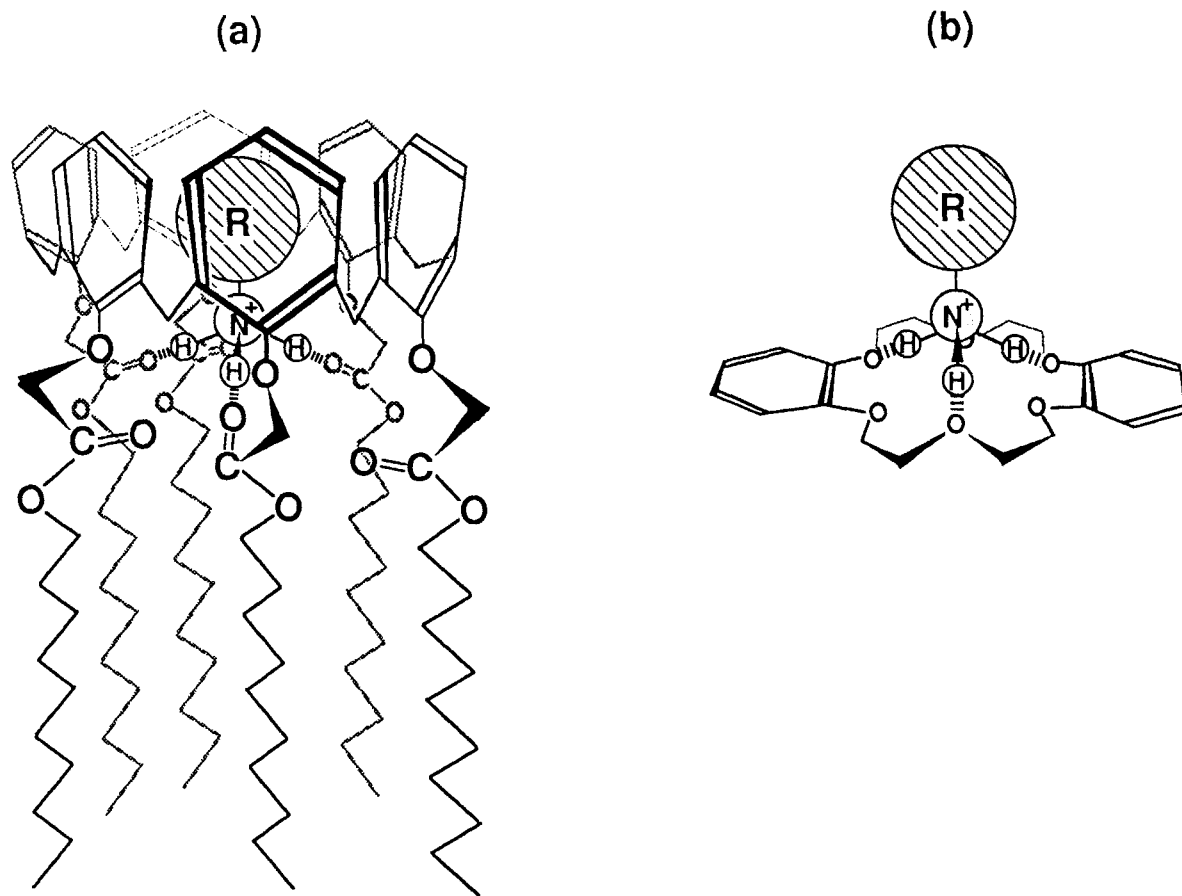
Figure 10 Schematic representations of the complexation geometries of host-guest complexes. (a) Possible geometry of the host-guest complex between calix[6]arene hexaester **8** and a protonated primary amine guest. (b) Plausible geometry of the host-guest complex between dibenzo-18-crown-6 (**9**) and a protonated primary amine guest.

(a)   (b)
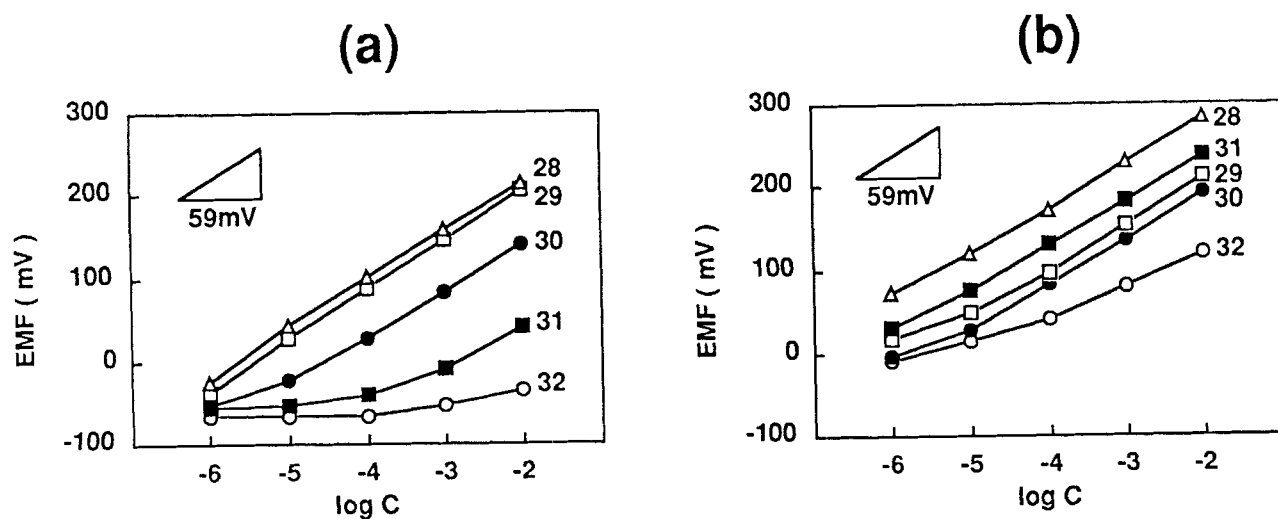
Figure 11 Potential vs concentration curves for simple primary amines at pH 7.0. (a) Electrode **8** containing calix[6]arene hexaester **8** as a sensory element. (b) Electrode **9** containing dibenzo-18-crown-6 (**9**) as a sensory element. Measured in 0.1 M Tris-HCl buffer (pH 7.0) at room temperature (*ca.* 20°C)[21].

trolled by simple lipophilicity of the guests; the response was in the order of guest **28** > **31** ≥ **29** ≥ **30** >> **32**. In contrast, a quite different potentiometric selectivity was

observed for electrode **8**; the response was in the order of guest **28** ≥ **29** >> **30** >> **31**, **32** (Figure 11a, Table II). The strongest responses were observed for the guests
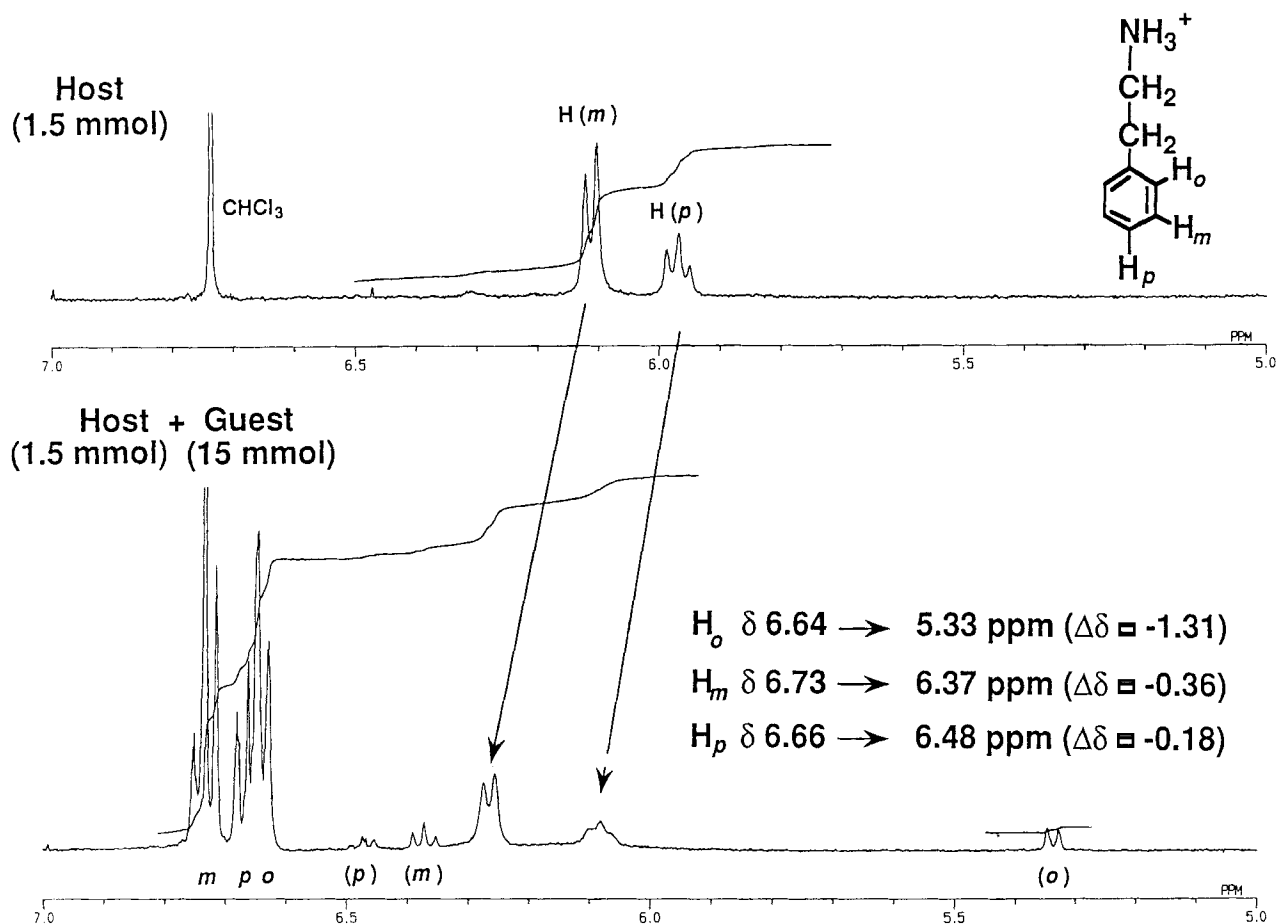
Figure 12 Changes in ^{1}H-NMR spectra by the host-guest complexation between calix[6]arene hexaester **8** and 2-phenylethylamine hydrochloride (**29•HCl**) in $CDCl_3$-CD_3OD (90:10) at an ambient temperature of 24 ± 1°C. Upper spectrum: [**8**] = 1.5 × 10^{-3} M. Lower spectrum: [**8**] = 1.5 × 10^{-3} M, [**29•HCl**] = 1.5 × 10^{-2} M. The signals corresponding to the *meta* and *para* protons of the aromatic ring of the host are indicated as H(*m*) and H(*p*), respectively. The signals corresponding to the *ortho*, *meta*, and *para* protons of the aromatic ring of the complexed and uncomplexed guest are indicated with parenthesized and unparenthesized abbreviations, respectively.

that have no bulky substituent at the α-carbon adjacent to the NH_3^+ group (**28, 29**). Guest **30** having a bulky substituent at the α-carbon gave a weaker response. On the other hand, the two bulkiest guests (**31, 32**) with a tertiary carbon adjacent to the NH_3^+ group gave very weak responses, which were actually almost the same as those of the electrode containing no host (figure not shown).[21]

The same tendency of potentiometric selectivity was observed for a series of catecholamines (**33~35**). Here again, by far the strongest response was observed for dopamine (**33**) having an unhindered structure as compared to noradrenaline (**34**) and adrenaline (**35**) having a substituent at the β-carbon and/or at the amino nitrogen. In addition, the discrimination between the unhindered and hindered guests was much more distinct in the calixarene electrode as compared to the crown ether electrode. The potentiometric selectivity coefficients ($K^{pot}_{i,j}$) for these guests are also listed in Table II.[21]

All of the potentiometric selectivities described so far are consistent with a discrimination of guests by inclu-

sion into the calixarene cavity. To obtain information on the geometry of the host-guest complex, a ^{1}H-NMR study was carried out by using 2-phenylethylamine hydrochloride (**29•HCl**) as a guest. Figure 12 shows the spectra of host **8** alone and a 1:10 mixture of the host and guest, respectively, in a 90:10 (v/v) mixture of $CDCl_3$ and CD_3OD. In the presence of the host, the signals of the complexed guest separated from those of the uncomplexed guest and shifted to the upfield direction (the lower spectrum in Figure 12). Large upfield shifts, especially of the *ortho* proton signal (δ 6.64→5.33 ppm, $\Delta\delta$ = -1.31 ppm), strongly support the formation of a host-guest complex with the aromatic part of the guest accommodated within the cavity of the host.[21]

The potentiometric selectivities, together with the NMR results, strongly support the potentiometric discrimination by inclusion of guests into the cavity of host **8**. The guests that are not hindered around the amino group would form stable inclusion complexes with a strong tripodal hydrogen bonding as shown in Figure

10a, which result in strong potentiometric responses. On the other hand, in the case of the guests having a hindered structure around the amino group, the complex formation would be weaker, leading to weaker potentiometric responses. Such a mode of discrimination cannot be expected for a simple 18-crown-6 ether (**9**) having no cavity for inclusion of organic guests. In conclusion, in addition to the potentiometric discrimination based on the recognition of specific functional groups such as charged groups or hydrogen bonding groups, the recognition of steric shapes of nonpolar moieties have been demonstrated as a new mode of potentiometric discrimination, which is potentially a general and versatile principle applicable for discrimination of a wide range of organic guests.

EXPERIMENTAL SECTION

Materials. The syntheses of the long alkyl derivatives of macrocyclic polyamines (**1~4**),[7a,24] the cytosine-pendant triamine host (**5**),[17] and the neutral cytosine derivatives (**6,7**)[17,20] have been described elsewhere. The calix[6] arene hexaester (**8**)[21] was synthesized by employing the reported procedure.[25]

Potential Measurements. The membrane compositions (wt % of sensory element, membrane solvent, and polymer matrix) of electrodes 1~9 were as follows: Electrode 1, **1**:DOP:PVC = 3:75:22;[7c] electrode 2, **2**:DOP:PVC = 3:75:22,[7a–c] **2**:NPOE:PVC = 1:79:20,[14] **2**:NPOE:PVC = 1.5:73:25.5,[16] or **2**:DBS:PVC = 2:70:28;[17] electrodes 3 and 4, (satd solution of **3** or **4** in NPOE):PVC = 74:26;[16] electrode 5~7, (**5**, **6** or **7**):DBS:PVC = 2:70:28;[17] electrode 1+6, **1**:**6**:DOP:PVC = 2.7:4.6:71.3:21.4 (molar ratio of **1** and **6** = 1:1);[18] electrode 3+6, (satd solution of **3** in DOP):**6**:PVC = 73.5:4.4:22.1;[18] electrodes 8 and 9, (**8** or **9**): DOS:PVC = 5:68:27 and 2:66:32, respectively.[21] See the respective references for the details. The cell assemblies were as follows: Ag | AgCl | 0.1 M KCl + 0.001 M TPA⁺Cl⁻ | membrane | sample solution ‖ 0.1 M K_2SO_4 ‖ 3 M KCl | AgCl | Ag (electrodes 1~7, 1+6, 3+6);[7a–c,16–18] Ag | AgCl | 0.01 M KCl + acetate buffer (pH 4.9) | membrane | sample solution ‖ 1 M LiOAc ‖ 3 M KCl | AgCl | Ag (electrodes 2);[14] Ag | AgCl | 0.01 M KCl | membrane | sample solution ‖ 0.1 M LiOAc ‖ 3 M KCl | AgCl | Ag (electrodes 8 and 9).[21] Abbreviations: DOP, dioctyl phthalate (bis(2-ethylhexyl) phthalate); NPOE, 2-nitrophenyl octyl ether; DBS, dibutyl sebacate; DOS, dioctyl sebacate (bis(2-ethylhexyl) sebacate); TPA⁺Cl⁻, tetrapentylammonium chloride. The potentiometric selectivity coefficients ($K_{i,j}^{pot}$) were determined at room temperature (*ca.* 20 °C)[7a,b,21] by the matched potential method in mixed solutions according to Gadzekpo and

Christian[26] or by the separate solution method according to Srinivasan and Rechnitz[27] (see also ref 28).

¹H-NMR Measurements. The ¹H-NMR experiments for the host-guest complex between calix[6]arene hexaester (**8**) and 2-phenylethylamine hydrochloride (**29•HCl**) were conducted by using tetramethylsilane (neat) as an external standard to validly evaluate the host-induced changes in the chemical shifts of the guest proton signals.[21]

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